A METHOD OF DETECTION OF PREDISPOSITION TO HIGH ALTITUDE PULMONARY EDEMA (HAPE)

FIELD OF INVENTION

The present invention relates to a method for the detection of predisposition to high altitude pulmonary edema (HAPE). It particularly relates with the allelic variants of iNOS (inducible nitric oxide synthase) gene, which has been found to be related with the prevalence of HAPE.

BACKGROUND OF THE WORK:

High altitude pulmonary edema (HAPE) is a form of noncardiogenic pulmonary edema that develops in approximately 10% of randomly selected mountaineers within 24h after rapid ascent to altitude above 4,000 m. A similar phenomenon is observed in the lowlander inductees to a height above 3000 m for various business reasons. An even higher incidence rate of about 60% has been demonstrated in subjects who are susceptible to HAPE as documented by previous occurrence of the disease (Houston CS et al 1960, Bartsch P et al 1997,1990). HAPE can be effectively prevented by prophylactic use of vasodilators or slow ascent. Nevertheless, it remains the most common cause of death related to high altitude exposure during trekking or mountaineering (Hackett PH et al 1990). The morbidity rate in Himalayan mountaineers was estimated to be 50% if immediate treatment with supplemental oxygen or rapid descent is impossible (Lobenhoffer HP et al 1982). Observed differences in clinical presentations and severity of the disease between racial and ethnic groups together with familial clustering favor a significant hereditary predisposition to the disease.

Although knowledge of the factors influencing the development of HAPE is still incomplete, there is experimental evidence that an exaggerated hypoxic pulmonary vasoconstriction (HPV) plays an important role (Scherrer U et al 1996). An excessive rise in pulmonary artery pressure has been demonstrated by invasive and noninvasive measurements at high altitude in individuals with HAPE. The uneven vasoconstriction in the capillaries sometimes results in "capillary leakage" followed by edema formation (Bartsch P et al 1991). Human subjects who are susceptible to the

disease demonstrate an increased pulmonary vascular response even during a brief exposure of high altitude. The underlying pathophysiological mechanism for this exaggerated HPV is still unknown. There is, however, evidence that the endogenous vasodilator nitric oxide (NO) modulates vascular reactivity (Palmer RMJ et al 1987). Regulation of vascular tone by NO is attributed to the intermediates of cGMP pathway (Bellamy TC et al 2002).

The following studies emphasize the involvement of NO in HAPE:

NO exerts its effect mainly via improvement of ventilation/perfusion ratio and lowering of alveolar to arterial oxygen tension difference by increasing arterial oxygen saturation (Scherrer U et al 1996). However, in the healthy volunteers, administration of the NO synthesis antagonist N^G-monomethyl-L-arginine (L-NMMA) during hypoxia increases pulmonary artery pressure and vascular resistance which is similar to that observed in HAPE. Due to this NO has been used as an inhalation therapy for the treatment of HAPE in the affected individuals (Anand IS et al 1998).

Phosphodiesterase 5 is the key enzyme responsible for cGMP hydrolysis in the lungs. The inhibitors of Phosphodiesterase 5 have been found to inhibit hypoxia induced pulmonary hypertension (Goldstein I et al 1998). Hypoxia decreases exhaled NO in mountaineers susceptible to HAPE indicating decreased NO production in such cases (Busch et al 2001). Thus defective NO synthesizing machinery imparting lower NO level may be envisaged to be responsible for the pathogenesis of HAPE. NO is synthesized by three isozymes nNOS (neuronal nitric oxide synthase, NOS1), iNOS (inducible nitric oxide synthase, NOS2) and eNOS (endothelial nitric oxide synthase, NOS3) (Michel T et al 1997). NOS1 and NOS3 are constitutively expressed while NOS2 is expressed upon induction. Among these the best candidate which is supposed to be defective in HAPE is eNOS (endothelial nitric oxide synthase) while induction of iNOS (inducible nitric oxide synthase) seems to be inevitable for the immediate recovery of the total NO reserve (Xia Y et al 1998). Moreover, robust cell signaling mechanisms generally favor the recruitment of inducible genes for immediate early physiological responses. It can be speculated that a defect in iNOS which doesnot permit its activation may not recover the reduced NO level in individuals exposed to hypoxia resulting in HAPE. The defect in iNOS may occur at genetic level in HAPE patients. In numerous cases, the expression of the genes has been found to get altered by the polymorphisms in the gene sequence (Qadar Pasha MA et al

2001). Hence, it is always possible that polymorphism in iNOS gene may alter its expression and associates with the disease.

Current status of the treatment of HAPE:

1. NO therapy: NO is being used as an inhalation therapy for the treatment of HAPE. It exerts its effect mainly via improvement of ventilation/perfusion ratio and lowering of alveolar to arterial oxygen tension difference by increasing arterial oxygen saturation.

NO induced improvement in arterial oxygenation in subjects with HAPE was accompanied by a shift in blood flow in the lung away from edematous segments and toward nonedematous segments results in evening/homogeneity of the vasoconstriction throughout the capillaries (Scherrer U et al 1996, Anand IS et al 1998).

2. Rapid descent: Rapid descent of HAPE patients not only prevents the worsening but even improves the pathogenesis of the disease (Hackett PH et al 2001).

3. Portable Air Chambers (PACs): PACs in the form of small cylinders filled with oxygen is often used as inhalation therapy for HAPE (Hackett PH et al 2001).

4. Genetic predisposition: The only study in this context suggests that genetic variation in endothelial nitric oxide synthase gene (eNOS) and angiotensin converting enzyme gene (ACE) may predispose individuals to HAPE (Droma Y et al 2002). The results are as follows:

	Controls	Patients
Glu298Asp (eNOS)	9.8%	25.6%
B/A (eNOS)	6.9%	32.2%
I/D (ACE)	4%	22%

Limitations of the available therapies for HAPE:

1. HAPE patients do not found to have homogenous response to NO inhalation. Moreover, concentration of required NO varies with the severity of the disease. Sometimes inadequate inhalation results in hypotension or even septic shock to the patients.
2. Immediate descent of the HAPE patients often remains impossible due to severe weather and rugged terrain (Anand IS et al 1998, Hackett PH et al 2001).
3. Carriage of PACs sometimes appears to be not feasible due to overloading problem. Improved conditions of the disease are often temporary as removal of chambers renders the patient worse (Hackett PH et al 2001).
4. The reported polymorphisms associated with HAPE are not specific but have also been shown to be associated with the disorders like diabetes, coronary artery disease, hypertension and myocardial infarction where elevated blood pressure is observed (Monti LD et al 2003, Via M et al 2003). The allelic frequency difference mentioned appears to be the same with other diseases. Hence the possibility of allelic contribution to the disease may be due to other related pathophysiologies like hypertension, which involves the exacerbations of HAPE. Moreover, the study does not include HA natives (high landers), a population residing blissfully in the same environment where the disease occurs.

Novelty of the invention is in providing a novel method for the detection of predisposition to HAPE.

Still another novelty is for providing a novel marker region in iNOS gene.

Still another novelty is for providing a novel SNP in iNOS gene.

Still another novelty is to demonstrate association of the allelic variants of iNOS gene with HAPE.

Another novelty is to provide novel primers and probes for amplification, which contains the novel SNP.

OBJECTS OF THE INVENTION:

Main object of the present invention is to provide a method for the detection of predisposition to HAPE, which obviates the limitations listed above.

Still another object is providing a novel SNP in iNOS gene.

Another object is to provide novel primers and probes for amplification, which contains the novel SNP.

One more object is to perform association analysis for the allelic variants between low landers and HAPE patients so that the relation with the disease could be scored.

SUMMARY OF THE INVENTION:

The present invention relates to the method of detection of predisposition to HAPE. It particularly relates with the allelic variants of iNOS gene, which has been related to the prevalence of HAPE. Defective Nitric Oxide (NO) synthesizing machinery imparting lower NO level has been envisaged to be responsible for the pathogenesis of HAPE. iNOS gene has been shown to be responsible for NO production as the inhibitors of NO production increased the severity of HAPE. Present invention provides a method for detection of predisposition to HAPE as the novel allelic variants of iNOS gene in the disclosed marker region was shown to be negatively associated with the prevalence of HAPE in a population.

Accordingly, present invention provides a method of detection of predisposition to high altitude pulmonary edema (HAPE), which comprises:

1. Selecting study subjects by monitoring HAPE associated symptoms and extracting genomic DNA from leukocytes by known methods,
2. Computationally locating the marker region on the iNOS gene in the functional region as given in SEQ ID No.1,
3. Designing and synthesizing specific nucleotide primers of SEQ ID No.2,which is the forward primer and SEQ ID No. 3, which the reverse primer and PCR amplifying the marker region in the iNOS gene ,
4. Sequencing the PCR product and identifying computationally the sequence variations (novel single nucleotide polymorphism) by comparison with the already existing sequence of human iNOS gene,
5. Screening the high altitude native population (HA natives), low lander inductees (HAPE controls) and low lander HAPE patients for the novel single nucleotide polymorphism, using above said primers of sequence SEQ ID No. 2 and SEQ ID No. 3.

6. Computing the frequencies of AA, AG and GG genotypes in the three said populations for establishing the association of the genotypes with HAPE.
7. Statistically analyzing the differences in the distribution of the allelic variants (AA, AG and GG genotypes) in the populations wherein GG genotype observed to be at low risk to HAPE and AA genotype at high risk of the disease.

In an embodiment, the primers suitable for the amplification of the iNOS region contain one or more polymorphic sites as in SEQ ID No:2 and SEQ ID No:3.

In another embodiment, the allelic variants of iNOS gene have AA, AG and GG genotypes,

Further, the invention provides a diagnostic kit for the detection of SNP genotypes comprising suitable primers and probes mentioned as SEQ ID NO: 2 and SEQ ID NO: 3.

In another embodiment of the invention the nucleic acid vectors used contain the allelic variants of the iNOS gene.

The allelic variants of human iNOS gene comprising the following single nucleotide polymorphism was found on comparison with the human iNOS gene sequence (Gene Bank Accession Number NT_010799).

Table 1

Site of change	Base change	Mutation type
19480	A/G	Transition

The invention also provides a method of analysing a nucleic acid from an individual for the presence of base at the polymorphic site shown in Table 1. This type of analysis can be performed on a plurality of individuals who are tested either for the presence or for the predisposition to HAPE. The susceptibility to the disease can then be established based

depending on the base or set of bases present at the polymorphic site in the individual tested.

The invention also provides oligonucleotide sequences (as listed in SEQ ID No: 2 and SEQ ID No: 3), suitable for use as primers and probes for the detection of polymorphic site mentioned in Table 1.

Further, a diagnostic kit comprising one or more primers or probes along with the required buffers and accessories suitable for identification of iNOS allelic variants to establish an individual's susceptibility to HAPE is also included in the invention.

Eukaryotic vectors comprising a DNA sequence coding for a protein or a peptide according to the invention are new materials and are also included in the invention. Host cells, for example, cloned human cell lines, can be transformed using the new vectors and are also included in the invention.

DESCRIPTION OF ACCOMAPNYING FIGURES/DRAWINGS

Figure 1 Schematic representation of the gene of inducible Nitric Oxide Synthase (iNOS) localization: 17 cenq$^{11.2}$. The vertical bars showing the exonic regions (From Gene bank Nucelotide Sequence ID No. NT_010799).

Figure 2 shows sequence file of the individual with AA homozygote.

Figure 3 shows sequence file of the individual with GG homozygote.

Figure 4 shows sequence file of the individual with AG heterozygote.

Figure 5 shows sequence file of the individual with TC heterozygote.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the preferred embodiments of the invention given for the purpose of disclosure. Alternative embodiments of the invention can be envisaged by those skilled in the art. All such alternative embodiments are intended to lie within the scope of this invention.

DESCRIPTION OF THE INVENTION:

The present invention relates to the method of detection of predisposition to HAPE. It particularly relates with the allelic variants of iNOS gene, which has been found to be related to the prevalence of HAPE.

I. Identification of the marker region on the iNOS gene:

Taking in consideration the important functions of NO at high altitude, iNOS, the inducible nitric oxide synthase gene was selected as the candidate gene for the study.

II. Selection of the study subjects:

Clinical severity of HAPE was assessed by Lake Louise acute mountain sickness (AMS) scoring system. Briefly, the patients were assessed for the presence of five symptoms: headache, gastrointestinal upset, fatigue, weakness, or both, dizziness, lightheadedness, or both, and difficulty in sleeping. Change in mental status, ataxia and peripheral edema were also assessed. Each of these symptoms were rated between 0 and 3. A score of 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. HAPE score is the sum of all 8 symptoms and patients were characterized by HAPE score>6 (Anand IS et al 1998). Lowlanders (LLs) were subjects who even after induction to high altitudes atleast thrice never found to have any of the above mentioned symptoms. High altitude (HA) natives were the permanent residents of HA from ancient times.

III. Extraction of genomic DNA from leukocytes:

Genomic DNA was extracted from blood using salting out method. Lysis of red blood cells in presence of high salt was followed by treatment with Nucleus lysis buffer (NLB). Proteins were precipitated and extraction of DNA was obtained in ethanol (Miller SA et al 1988).

IV. Identification of the allelic variants of the iNOS gene:

Novel polymorphism of the invention:

As a first step to the present invention, the applicants carried out the PCR amplification of marker region of the iNOS gene using self designed oligonucleotide primers. The primers were designed in accordance with the human iNOS gene sequence (Gene Bank Accession Number NT_010799). The sequencing of the purified PCR product revealed a novel single

nucleotide polymorphism in Intron 7 of the human iNOS gene. It was apparent, therefore that there is a hitherto unrecognized allele or subtype of the human iNOS gene.

The present invention provides a sequence for the allelic variants of human iNOS gene comprising the following novel single nucleotide polymorphism compared with the human iNOS gene sequence in the database.

For example, the nucleotide sequence of the allelic variant of human iNOS gene (SEQ ID NO: 1) having the polymorphic site listed in Table 1 may be-

5'CAGCGGAGTGATGGCAAGCACGACTTCCGGGTGTGGAATGCTCAGCT
CATCCGCTATGCTGGCTACCAGATGCCAGATGGCAGCATCAGAGGGGA
CCCTGCCAACGTGGAATTCACTCAGGTACCCGGCCCAGCCTCAGCC
A*/GCCGGCCATTGGGGCGGGGAGCCCCGTGGTGAGCGAGTGACAGAG
TGGAGCCCAGAGGAGACACGCAGCCCGGGCTTACAGACTCACAGGGCC
CGTCTTGTTCCCCAGCTGTGCATC3'

In the above sequence the SNP* is shown in bold.

V. Association Analysis with the disease

Analysis of the SNP in 42 HA natives, 39 HAPE controls and 18 HAPE patients revealed three genotypes, namely AA, AG and GG. The distribution of alleles is summarized in Table 2.

Table 2

Study subjects	A	G
HAPE controls (n=39)	0.35	0.65
HAPE patients (n=18)	0.58	0.42
HA natives (n=42)	0.18	0.82

The frequency of the G allele was found to be in the order of HA natives>HAPE controls>HAPE subjects. The biostatistical analysis showed a significant association of G allele with HA adaptation and A allele with the disease as mentioned in Table 3.

Herein the odds ratio (OR) and 95% confidence of interval was used as a measure of the strength of the association between genotypic combination and the disease. P value of <0.05 was considered statistically significant.

Table 3

Association type	χ^2 value	p value	Odds ratio	95% CI	Relative risk
HAPE patients & HAPE controls	10.63	0.001	2.56	1.45-4.54	1.66 (1.21-2.27)
HAPE patients & HA natives	33.96	<0.001	6.29	3.30-12.01	3.22 (2.05-5.06)
HAPE controls & HA natives	7.42	0.006	-	-	-

Nitric oxide synthase for its reaction to synthesize nitric oxide, requires oxygen which acts as a cofactor in the reaction. Oxygen binds to the oxygenase domain in iNOS and contributes to the synthesis of NO. In hypoxic condition scarcity of oxygen may lead to lower NO production, however any modification in the oxygenase domain, which modify the activity of the enzyme in such a way that it requires no oxygen or less oxygen may contribute to normal NO production. NO improves oxygenation of hemoglobin and normal NO production may involve the mechanisms acting in acclimatization, hence any alteration in oxygenase domain may be favorable for the production of NO. In the present investigation the novel SNP found in intron 7 is present near to the oxygenase domain of NOS2 gene which spans exon 7 to exon 16. It is quite possible that the SNP found is in linkage disequilibrium to a nearby SNP, which is contributing to the final impact on NO production by NOS2 gene.

VI. Diagnostic kits

The invention further provides diagnostic kit comprising at least one or more allele specific oligonucleotides as described in SEQ ID 2 and 3. Often, the kits contain one or more pairs of allele-specific oligonucleotides hybridizing to different forms of a polymorphism. In some kits, the allele-specific oligonucleotides are provided immobilized to a substrate. For

example, the same substrate can comprise allele-specific oligonucleotide probes for detecting at least the polymorphism shown in Table1. Optional additional components of the kit include, for example, restriction enzymes, reverse transcriptase or polymerase, the substrate nucleoside triphosphates, means used to label (for example, an avidin enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for reverse transcription, PCR, or hybridization reactions. Usually, the kit also contains instructions for carrying out the methods.

VII. Nucleic acid vectors

Variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer, which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage promoters, glycolytic enzyme promoters and tRNA promoters, depends on the host selected. Commercially available expression vectors can also be used. Suitable host cells include bacteria such as E.coli, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell lines and derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide.

The invention further provides transgenic non-human animals capable of expressing an exogenous variant gene and/or having achieved by operably linking the gene to a promoter and optionally an enhancer, and microinjecting the construct into a zygote. Inactivation of endogenous variant genes can be achieved by forming a transgene in which a cloned variant gene is inactivated by insertion of a positive selection marker. The transgene is then introduced in to an embryonic stem cell, where it undergoes homologous recombination with an endogenous variant gene. Mice and other rodents are preferred animals. Such animals provide useful drug screening systems.

Accordingly, the main embodiment of the present invention relates to a method for detecting predisposition to high altitude pulmonary edema (HAPE), said method comprising the steps of:

(a) selecting study subjects by monitoring high altitude pulmonary edema associated symptoms,

(b) extracting genomic DNA from leukocytes by conventional methods from the study subjects,

(c) amplifying Intron 7 of the human iNOS gene of SEQ ID No.1 by designing and synthesizing Forward and Reverse oligonucleotide primers of SEQ ID No. 2 and SEQ ID No. 3, respectively,

(d) identifying computationally novel Single Nucleotide Polymorphism (SNP) by comparing with the already existing sequence of human iNOS gene,

(e) screening the high altitude native population (HA natives), low lander natives (HAPE controls) and low lander HAPE patients for the novel single nucleotide polymorphism, using above said primers of SEQ ID No. 2 (Forward Primer) and SEQ ID 3 (Reverse Primer),

(f) computing the frequencies of AA, AG and GG genotypes in the populations of step (e) for establishing the association of the genotypes with high altitude pulmonary edema, and

(g) predicting and statistically analyzing the differences in the distribution of the allelic variants (AA, AG and GG genotypes) in the populations wherein GG genotype at 19480 position are at low risk to high altitude pulmonary edema and AA genotype at 19480 position are at high risk of the disease.

Another embodiment of the present invention relates to the oligonucleotide primers capable for amplification of Intron 7 of human iNOS gene are selected from group comprising of

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

Yet another embodiment of the present invention relates to the oligonucleotide primers contain one or more polymorphic sites selected group comprising of

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer, and

(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer.

Still another embodiment of the present invention relates to the allelic variants wherein the allelic variants of the of iNOS gene have AA, AG and GG genotypes

A diagnostic kit for the detection of SNP genotypes having predisposition to high altitude pulmonary edema (HAPE) said kit comprising of primers and probes:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer
(b) 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

One more embodiment of the present invention relates to the Primers suitable for amplification of iNOS gene region containing one or more polymorphic sites, said primers include:

(a) 5' CAG CGG AGT GAT GGC AAG CAC GAC 3' (SEQ ID No.2), which is a forward primer
(b) SEQ ID 3: 5' GAT GCA CAG CTG GGG AAC AAG ACG 3' (SEQ ID No.3), which is a reverse primer

In another embodiment of the present invention relates to the nucleic acid vectors containing the allelic variants of the iNOS gene.

The following examples are given by way of illustration of the present invention and should construe to limit the scope of the present invention.

EXAMPLES

EXAMPLE 1

Identification of the marker gene:

Taking in consideration the important functions of NO at HA, iNOS, the inducible nitric oxide synthase was selected as the candidate gene for the study.

EXAMPLE 2

Selection of the study subjects:

Clinical severity of HAPE was assessed by Lake Louise acute mountain sickness (AMS) scoring system. Briefly, the patients were assessed for the presence of five symptoms: headache, gastrointestinal upset, fatigue, weakness, or both, dizziness, lightheadedness, or

both, and difficulty in sleeping. Change in mental status, ataxia and peripheral edema were also assessed. Each of these symptoms were rated between 0 and 3. A score of 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. HAPE score is the sum of all 8 symptoms and patients were characterized by HAPE score>6 (Anand IS et al 1998). LLs were subjects who even after induction to high altitudes atleast thrice never found to have any of the above mentioned symptoms. HA natives were the permanent residents of HA from ancient times.

EXAMPLE 3

Extraction of genomic DNA from leukocytes:

Genomic DNA was extracted from blood using salting out method. Lysis of red blood cells in presence of high salt was followed by treatment with Nucleus lysis buffer (NLB). Proteins were precipitated and DNA was extracted from peripheral blood leukocytes using a modification of the salting out procedure. The concentration of the DNA was determined by measuring the optical density of the sample, at a wavelength of 260 nm. (Miller SA et al 1988).

EXAMPLE 4

Identification of the allelic variants of the iNOS gene:

This example describes the identification of allelic variants of iNOS gene by PCR and sequencing using certain oligonucleotide primers according to the invention. The DNA was then amplified by polymerase chain reaction by using the oligonucleotide primers:

1. 5'CAG CGG AGT GAT GGC AAG CAC GAC 3'(as listed in SEQ ID NO:2) and
2. 5' GAT GCA CAG CTG GGG AAC AAG ACG 3'(as listed in SEQ ID NO:3).

Polymerase chain reaction was carried out using the following conditions:

Step 1 94^0C for 4 min
Step 2 94^0C for 30 sec
Step 3 62.5^0C for 30 sec
Step 4 72^0C for 45 sec
Step 5 34 times to Step 2
Step 6 72^0C for 10 min

PCR was performed in a Perkin Elmer GeneAmp PCR System 9600. This reaction produced a DNA fragment of 258bp when analyzed by 2% agarose gel electrophoresis. The PCR product was purified from band cut out of agarose gel using a Amersham Pharmacia gel extraction kit (Amersham) and both the strands of the PCR product were directly sequenced using dye terminator chemistry on an ABI Prism 377 automated DNA sequencer. The PCR product was identical to the human iNOS gene sequence except of the novel single base pair change mentioned in Table1.

EXAMPLE 5

Nucleotide sequence of the Allelic Variant of the iNOS gene:

The nucleotide sequence of the allelic variant of iNOS gene derived using the method as described in example 1-

5'CAG CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT GCC AAC GTG GAA TTC ACT CAG GTA CCC GGC CCA GCC TCA GCC A*/GCC GGC CAT TGG GGC GGG GAG CCC CGT GGT GAG CGA GTG ACA GAG TGG AGC CCA GAG GAG ACA CGC AGC CCG GGC TTA CAG ACT CAC AGG GCC CGT CTT GTT CCC CAG CTG TGC ATC 3'

In the above sequence the SNP* is shown in bold.

EXAMPLE 6

G allele is related with adaptation and A allele associates with the disease:

A method as described in example 4 is applied to a series of DNA samples extracted from HA natives, HAPE controls and HAPE patients. A highly significant association of G allele with the HA adaptation and A allele with the disease has been observed. The results are summarized in the table below:

Association type	χ^2 value	p value	Odds ratio	95% CI	Relative risk
HAPE patients & HAPE controls	10.63	0.001	2.56	1.45-4.54	1.66 (1.21-2.27)
HAPE patients & HA natives	33.96	<0.001	6.29	3.30-12.01	3.22 (2.05-5.06)
HAPE controls & HA natives	7.42	0.006	-	-	-

Hence, individuals with GG genotype being at low risk and those with AA genotype being at high risk for HAPE, can be expected to hold true for other populations also.

EXAMPLE 7

Nucleic acid vectors containing the iNOS variant sequences:

Vectors and host cells transformed with the allelic variants of the iNOS gene containing one or more polymorphic sites as listed in table 1, can be prepared, for example, as detailed below.

Variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer, which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage, glycolytic enzyme and tRNA, depends on the host selected. Commercially available expression vectors can also be used. Suitable host cells include bacteria such as E.coli, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell

lines and derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide.

Advantages of the present invention:

The present invention adds following points to the treatment of HAPE.

1. Inducible nitric oxide synthase gene as a novel marker for HAPE studies.
2. Novel primer sequences responsible for the amplification of PCR product containing novel SNP.
3. Novel SNP (19480 A/G) that can be used for further association studies.
4. A significant association of wild type allele (A) to the disease (Table 2 and 3).
5. A significant association of mutant allele (G) to adaptation (Table 2 and 3).
6. A significant difference between the frequency of alleles with respect to HA native and HAPE controls (Table 2 and 3).
7. The presence of G allele predisposes an individual to less chances of getting diseased.
8. It may help individuals to decide visiting high altitude for various reasons.

Provided below is the sequence listing information for SEQ ID Nos. 1, 2 and 3

SEQUENCE LISTING

GENERAL INFORMATION

APPLICANT: CSIR

TITLE OF INVENTION: Method for the detection of predisposition to high altitude pulmonary edema (HAPE).

NUMBER OF SEQUENCES: 03

CORRESPONDING ADDRESS: Institute of genomics and integrative biology, CSIR, Delhi University Campus, Mall Road-110007, India.

Telephone: +91-11-27666156 Fax: +91-11-27667471

INFORMATION FOR SEQUENCE ID NO: 1

1. SEQUENCE CHARACTERISTICS

1. LENGTH: 258 bp

2. TYPE: DNA

5'CAG CGG AGT GAT GGC AAG CAC GAC TTC CGG GTG TGG AAT GCT CAG CTC ATC CGC TAT GCT GGC TAC CAG ATG CCA GAT GGC AGC ATC AGA GGG GAC CCT GCC AAC GTG GAA TTC ACT CAG GTA CCC GGC CCA GCC TCA GCC **A*/G**CC GGC CAT TGG GGC GGG GAG CCC CGT GGT GAG CGA GTG ACA GAG TGG AGC CCA GAG GAG ACA CGC AGC CCG GGC TTA CAG ACT CAC AGG GCC CGT CTT GTT CCC CAG CTG TGC ATC 3'

3. ORGANISM: *Homo sapiens* (Humans)

4. IMMEDIATE SOURCE: PCR

5. NAME/KEY: Marker Region

6. SEQUENCE ID # 1

INFORMATION FOR SEQUENCE ID NO: 2

1. SEQUENCE CHARACTERISTICS

LENGTH: 24 bp

TYPE: DNA
5'CAG CGG AGT GAT GGC AAG CAC GAC 3'

ORGANISM: Artificial sequence

IMMEDIATE SOURCE: Synthetic

NAME/KEY: Synthetic Oligonucleotide

SEQUENCE ID # 2

INFORMATION FOR SEQUENCE ID NO: 3

1. SEQUENCE CHARACTERISTICS

LENGTH: 24 bp

TYPE: DNA
5' GAT GCA CAG CTG GGG AAC AAG ACG 3'

ORGANISM: Artificial sequence

IMMEDIATE SOURCE: Synthetic

NAME/KEY: Synthetic Oligonucleotide

SEQUENCE ID # 3

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